

Drosophila Geotaxis as a Tool for the Study of Aging

by

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Abstract

Age-dependent changes in geotaxis profiles have been examined in 27 wild-type populations of Drosophila, representing a diversity of species (16), semispecies (4) and strains (7). In addition, four strains of D. melanogaster (two strains selected for postponed senescence and increased lifespan, and two control strains) have been tested. Tests were carried out at a minimum of three test ages, and involve the use of a calibrated, adjustable inclined plane that can be set at any angle between 0° and 85°. Among selected lines, decline in geotactic response occurs later in the long-lived flies than in the controls (28 days vs. 14 days). Longer-lived flies continue to show an increase in negative geotactic response through age 14-days. These results suggest that common processes may be influencing the rate of decline in geotactic response and longevity. Further analysis of the mechanisms underlying age-dependent changes in geotaxis may reveal factors which influence the aging process itself. The use of geotaxis aging markers in a broad range of Drosophila species reflecting varying degrees of genetic relatedness is proposed to test the universality vs. specificity of aging processes.

Introduction

Drosophila orientation to gravity, i.e., geotaxis, has been a valuable experimental system for addressing a broad range of questions concerning the aging process. As summarized in an earlier communication (Schnebel et al., 1986), age-dependent changes in geotactic response have provided biological markers for identifying senescent individuals, examining environmental effects on aging individuals, determining the physiological basis of behavioral decline, localizing age-dependent changes at the cellular and molecular levels that accompany such behavioral decline, and identifying genetic mutants which alter aging patterns (Herman et al., 1971; Miquel et al., 1976; 1979; Leffelaar and

Grigliatti, 1984a, b).

Recently, Johnson (1987) has shown that processes which contribute to aging can potentially be identified using behavioral markers in genetically manipulated strains of nematodes exhibiting differences in lifespan. Since Drosophila lifespan can also be altered by genetic selection (Luckinbill et al., 1984; Rose, 1984; Hoffmann and Grossfield, unpublished data), Drosophila geotaxis provides an appropriate behavioral system for similar investigations of aging processes.

Additionally, the question of whether "aging hypotheses" derived from genetically selected strains apply to other taxa, can be easily tested using the array of strains and species representing different degrees of genetic relatedness present in the genus Drosophila.

Materials and Methods

Animals and Housing

Twenty seven Drosophila populations representing a diversity of species (16), semispecies (4) and strains (7) were tested for age-dependent changes in geotactic response (for details, see Schnebel et al., 1986). Flies were housed at 19-20°C, 67-70%RH and constant light, and maintained on a raisin-based culture medium. In addition, four new strains of D. melanogaster (obtained from Dr. Robert Arking: Wayne State University) were also used in geotaxis experiments after undergoing special selection regimes for postponed senescence and increased lifespan (two selected strains and two control strains). These strains were maintained at 25°C and 60-70%RH.

Genetic Selection Procedures

Selection protocols followed those of Luckinbill et al. (1984). Through these procedures, two long-lived strains: NLA and NLB (NL designates non-density controlled, late-reproducing lines), and two control strains: RA and RB (R designates random, unselected control lines) were produced.

Geotaxis Experiments

Tests on the twenty seven populations of Drosophila followed procedures described in Schnebel et al. (1986). Briefly, flies aged 1-2 days, 7-9 days and 30-36 days were tested in glass tubes resting on an inclined plane that could be set at any angle between 0° and 85°. The number of flies in the upper and lower halves of the tube was recorded at each angle. Data reveal the minimum angle required to elicit a geotactic response (geotactic sensitivity), directional tendencies at all angles, and patterns of age-dependent loss of geotactic response.

Procedures for testing the four strains of D. melanogaster that underwent different selection regimes were modified as follows: Three hundred virgins of each sex were collected for each strain and tested separately. Thus, eight

groups of flies were tested for a total of 2400 flies. Groups: NLAm, NLAf, NLBm, NLBf, RAf, RBm, RBf. Flies were maintained in groups of 20/vial and transferred to fresh food vials three times/week. At the time of transfer, the number of dead and escaping flies was recorded. For each of the eight groups, ten numbered vials (200 flies) were used for housing experimental flies, while the remaining 100 individuals were maintained to replace any lost flies before experiments. Experimental vials were chosen randomly each day using numbers provided by a computerized random number generator.

Flies from the eight groups were tested on the day of eclosion (day 0) and on days 3, 7, 14, 21, 28, 35, 42, 49, 56 and 63 post eclosion. On each test day, two vials of each sex and strain were tested. The twenty flies from each test vial were transferred to a 30cm glass tube under light carbon dioxide anesthesia. Flies were set aside for a minimum of 30min before testing on the inclined plane. All tests were done in a "dark" chamber where the only light source was a flashlight covered with both uv and red filters. Recorded light levels during testing measured 0.22-0.28 footcandles. Flies were tested for geotactic response at angles ranging from 0° to 80° , and at three different orders of presentation (increasing angles: 0° to 80° ; decreasing angles: 80° to 0° ; and random presentation of angles). For testing, flies were dislodged to the bottom of the tube and the flashlight turned off before raising the board to a test angle. After 1min at the test angle, the flashlight was turned on and the number of flies in each half of the tube was recorded. Testing was done at normal rearing temperature and relative humidity.

Results were plotted as Gt values (Bean, 1977; Schnebel et al., 1986). This value can range from -1 to +1, where -1 indicates that for a specific angle, all flies are observed in the top half of the tube (negative geotaxis reflects movement away from the force of gravity). Conversely, a value of +1 results if all flies are in the bottom half of the tube. For preliminary analyses, results from each order of presentation of angles were pooled to provide mean Gt values at all test angles for each strain, sex and age.

Results and Discussion

Fig.1a compares geotactic responses in long-lived females (strain NLBf: LT50 = 52 days) and control females (strain RBf: LT50 = 45 days) at three test ages (0 days, 3 days, 14 days). Both strains show a similar increase in negative geotactic response through the first two test ages. However, by the third test age, RBf flies show a significant decline in response (paired t-test of Gt values at 3-days vs. 14-days: $t = -2.828$, $p < 0.025$), whereas the longer-lived NLBf flies become significantly more negatively geotactic (paired t-test of Gt values at 3-days vs. 14-days: $t = 4.027$, $p < 0.005$). Not only does negative geotactic response in the long-lived strain decline at a later age (28 days, not shown), but they continue to show an increase in negative geotaxis through age 14-days (the age at which the control strain has already shown a decline). Johnson (1987)

Fig 1a. Geotactic profiles of long-lived females (NLBf) and control females (RBf) at three test ages (0 days, 3 days, 14 days).

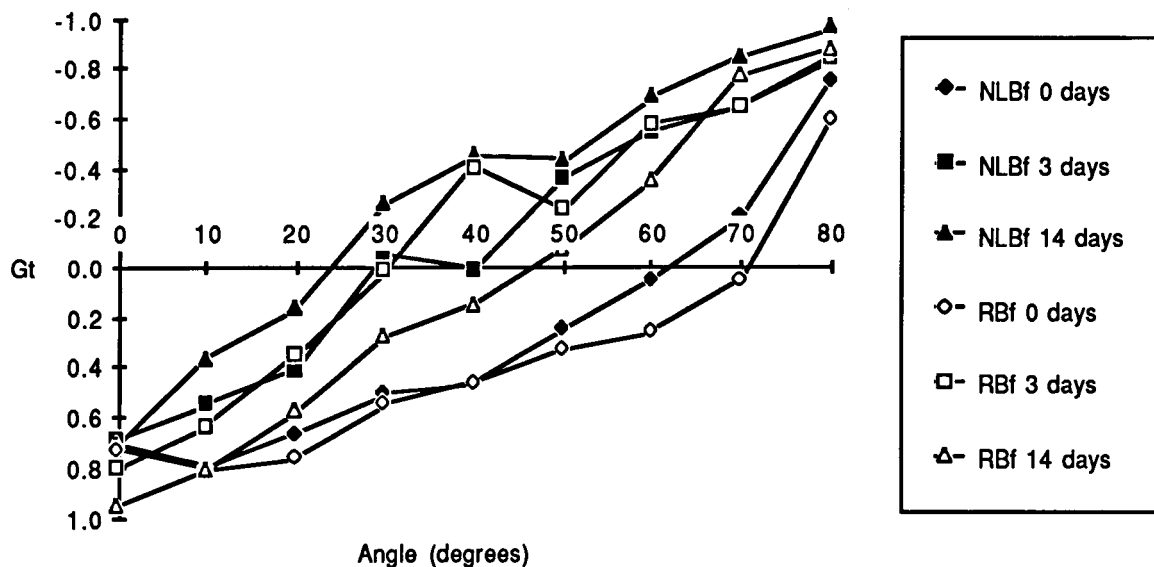
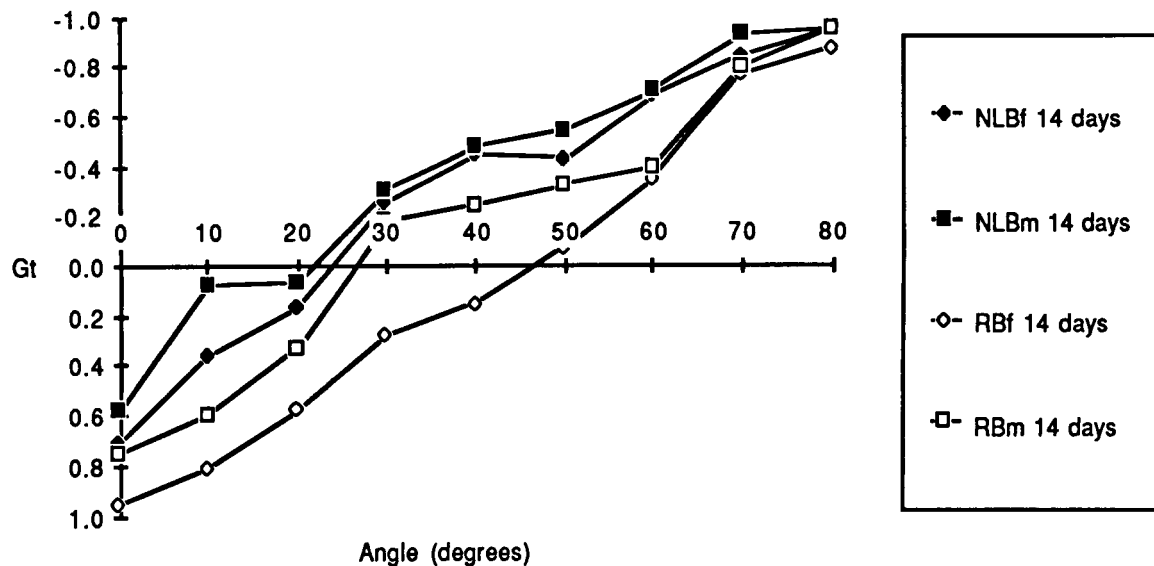


Fig. 1b. Geotactic profiles of long-lived females and males (NLBf and NLBm) vs. those of control females and males (RBf and RBm) at age 14 days.



suggests that the key criterion for choosing appropriate biomarkers of aging is to demonstrate that manipulations which prolong life also slow processes displaying age-related functional changes. He also suggests that when time of loss of function in a behavioral/physiological system covaries with lifespan, the two phenotypes may be specified by a common process or processes. Since geotactic response declines more slowly in the long-lived strain than in the short-lived control, common processes may be influencing the rate of decline in geotaxis and longevity. Similarly, comparisons of individuals with prolonged (NLBf) vs. "normal" (RBf) development of geotactic response, may ultimately reveal factors that affect the rate-determining processes which influence aging differences between those individuals.

Fig.1b shows that at the age where strain differences become apparent (day-14), long-lived males and females are more strongly negatively geotactic than control males and females. However, differences are most significant between females of the two strains (paired t-test of Gt values of NLBf vs. RBf: $t = -5.592$, $p < 0.0005$; NLBm vs. RBm: $t = -4.072$, $p < 0.005$), suggesting the use of females for the more detailed analyses of behavioral/physiological decline.

Figs. 2a (age: 1-2 days) and 2b (age: 7-9 days) compare the geotactic responses of five closely related strains and species of the D. melanogaster species group. These strains and species were maintained under identical conditions without any selection regime for lifespan. Each population has a similar negative geotactic response at the earlier test age. Yet, differences in the age-dependent patterns of change become evident at the older test age based on the following criterion: The angle at which the number of flies in the top half of the tube becomes significantly greater (Chi-square test) than the number of flies in the bottom half can be used as a measure of geotactic sensitivity (the smaller the angle necessary to elicit a significant geotactic response, the greater the geotactic sensitivity). The "critical angles" at the first/second test ages for each population are as follows: simulans-G: $15^{\circ}/10^{\circ}$, indicating a slight increase in sensitivity; mel-F: $20^{\circ}/25^{\circ}$ and ananassae: $10^{\circ}/15^{\circ}$, indicating slight decreases in sensitivity; mel+/: $15^{\circ}/65^{\circ}$, indicating a large decrease in sensitivity; simulans: $10^{\circ}/\text{none}$, indicating a decline in sensitivity to the extent that there is no longer a significant difference between the number of flies at the top and bottom of the tube at the second age. If the rate of loss in geotactic response can be correlated with differences in lifespan among these test populations, this would support the ideas that a) decline in geotaxis and decline in longevity may share some common causal process, and b) this process need not be specific to genetically manipulated populations, but instead, may be more universal. Such comparisons could be extended to different Drosophila species of varying degrees of genetic relatedness. This type of approach may reveal whether factors which influence aging and senescent decline in geotactic function are shared across taxa.

Fig.2a. Geotactic profiles of closely related strains in the *D. melanogaster* species group (Age:1-2 days).

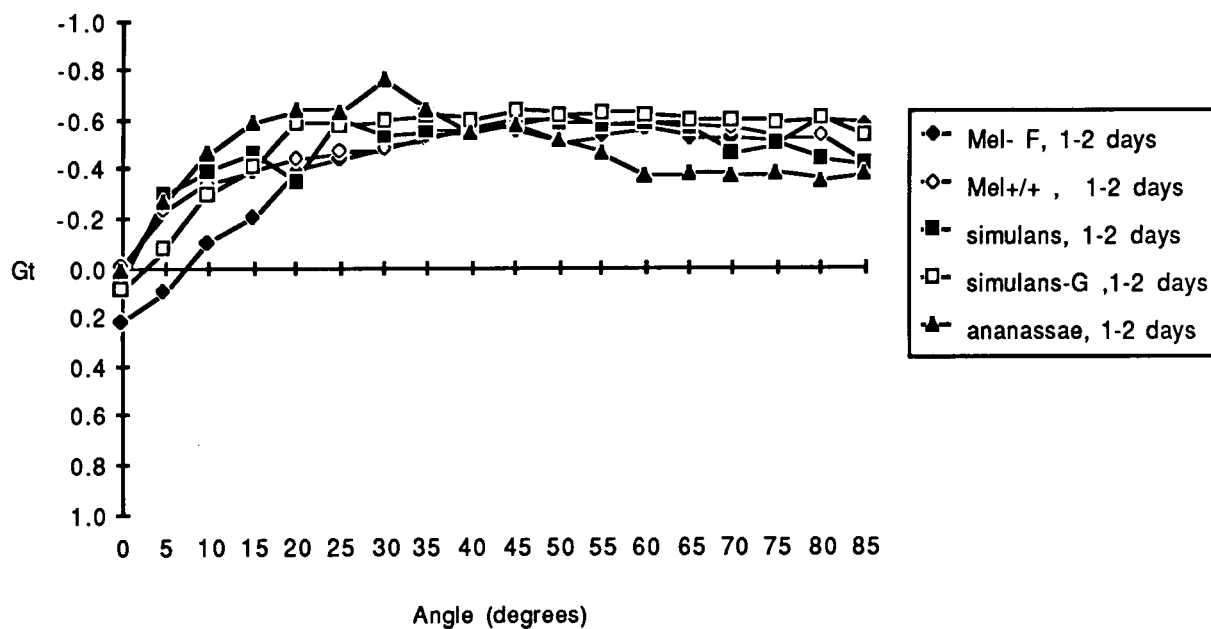
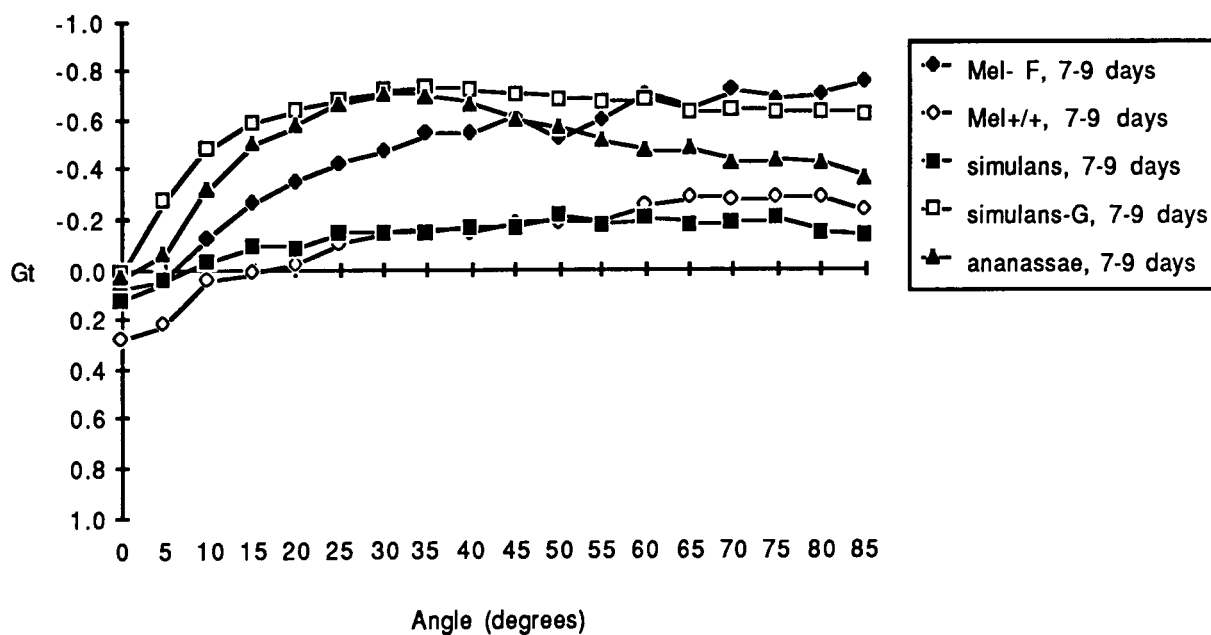


Fig. 2b. Geotactic profiles of closely related strains and species in the *D. melanogaster* species group (Age: 7-9 days).



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